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Award Number: W81XWH-11-1-0630

TITLE: Analysis of 3D Subharmonic Ultrasound Signals from Patients with Known Breast Masses for Lesion Differentiation

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REPORT DATE: October 2013

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2013	Annual Summary	30 September 2012 - 29 September 2013

4. TITLE AND SUBTITLE
Analysis of 3D Subharmonic Ultrasound Signals from Patients with Known Breast Masses for Lesion Differentiation

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0630

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Thomas Jefferson University
Philadelphia, Pennsylvania 19107

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of this award is to help the principle investigator transition into a long term career in breast cancer imaging research through both training and independent research. Over the second year of this project, the focus has begun to focus less on the training aspects and more towards the independent research phase. Work within the training phase has continued to include observation of an NIH funded multi-center breast imaging trial using contrast-enhanced 3D subharmonic imaging for the characterization of mammographically identified breast masses at attendance at two medical based conferences. Clinical training has also included time spent observing breast radiologists perform reads, and case and research conferences. Subharmonic videos continue to be obtained as part of a larger NIH funded clinical trial(currently with 132 total exams completed). The research component of this award has focused on using preliminary algorithms for improving vascular depictions of these lesions. Additionally, parametric imaging of these datasets and investigating spatial deviations appears to be a good basis for diagnosing malignancy. These algorithms have also been applied to existing contrast enhanced ultrasound exams of murine xenograft tumors to investigate correlations to immunohistochemical marker expression (a potentially useful clinical translation for monitoring treatment response).

15. SUBJECT TERMS
Breast Cancer, Ultrasound Imaging, Ultrasound Contrast Agent, Postdoctoral training

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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3 TABLE OF CONTENTS

4 INTRODUCTION

The purpose of this award is to help the principle investigator (PI) transition into a long term career in breast cancer imaging research through both training and independent research. While the focus of this project has shifted more towards the independent research, the clinical training component continues to include the observation of an NIH funded, multi-center breast imaging trial (data from which will be used in the research portion of this grant), time spent with radiologists specializing in breast imaging, and attendance at clinical research and case conferences. The research portion of this project is to develop computer-based analysis software that will extract physical parameters from a new method of ultrasound imaging (subharmonic imaging) to improve breast lesion characterization. These algorithms have also been applied to an existing contrast enhanced ultrasound dataset from murine xenografts to determine their relationship with immunohistochemical angiogenic marker expression (which may be useful as a potential tool for monitoring treatment response). Currently, mammography leads to an unacceptably high rate of false positive findings. Thus, the ultimate goal of this research is to develop subharmonic ultrasound image (SHI) processing algorithms to improve the classification of breast lesions.

5 BODY

5.1 Training Component

Work within the training arm has included the continued observation of an NIH funded multi-center breast imaging trial using contrast-enhanced 3D subharmonic imaging for the characterization of mammographically identified breast masses. The PI's involvement in the project has included assistance with drafting the protocols, gaining regulatory approval, testing the experimental software, observing data collection, and data analysis. To date, 102 patients have been observed at Thomas Jefferson University (TJU) and patient enrollment began in late September at the University of California, San Diego (34 subjects enrolled to date). The ability to assist in this process has allowed the PI to gain a better understanding of the requirements for starting and running a large scale breast imaging trial. Additionally, the data collected continues to be used for algorithm development as will be discussed in the research component below (section 5.2).

The PI has also had the opportunity to observe radiologists at TJU's breast imaging center as they interpret mammograms, breast ultrasounds, and breast MRIs. Additionally, this project has included attendance at TJU's breast case conferences, weekly Kimmel Cancer Center grand rounds, Department of Radiology seminars, and attendance at the 2012 Leading Edge in Diagnostic Ultrasound Breast Ultrasound Tutorial. Finally, the PI has also attended and presented at two leading medical conferences: The American Institute of Ultrasound in Medicine's Annual Meeting, and the World Molecular Imaging Congress. These presentations and training have helped the PI gain a larger picture of breast cancer care and emerging areas of research within the field. In light of this training, the PI has gained a much broader perspective on the diagnosis and management of cancer,

the role of radiology in this process, and the potential areas of improvement which breast ultrasound research may contribute to.

5.2 Research Component

The main focus of the research component in year 2 has been on the development of methods to quantify and visualize blood flow kinetics from subharmonic signals within breast lesions and to improve the visualization of blood vessels in 3D subharmonic ultrasound volumes. To date, data has been collected from 132 patients with mammographically identified breast lesions as part of NIH R01 CA140338. Image processing to date has focused on isolating regions of contrast flow in the three dimensional space to better isolate these regions. After data collection is complete, these improved depictions will be interpreted by blinded radiologists and compared to the unprocessed data sets to determine if these improvements offer significant clinical diagnostic values. Additionally, we have begun to use these measurements and their respective standard deviations as a potential quantifiable parameter for separating malignant vs. benign lesions. Time intensity curves were generated for each individual slice for each case.

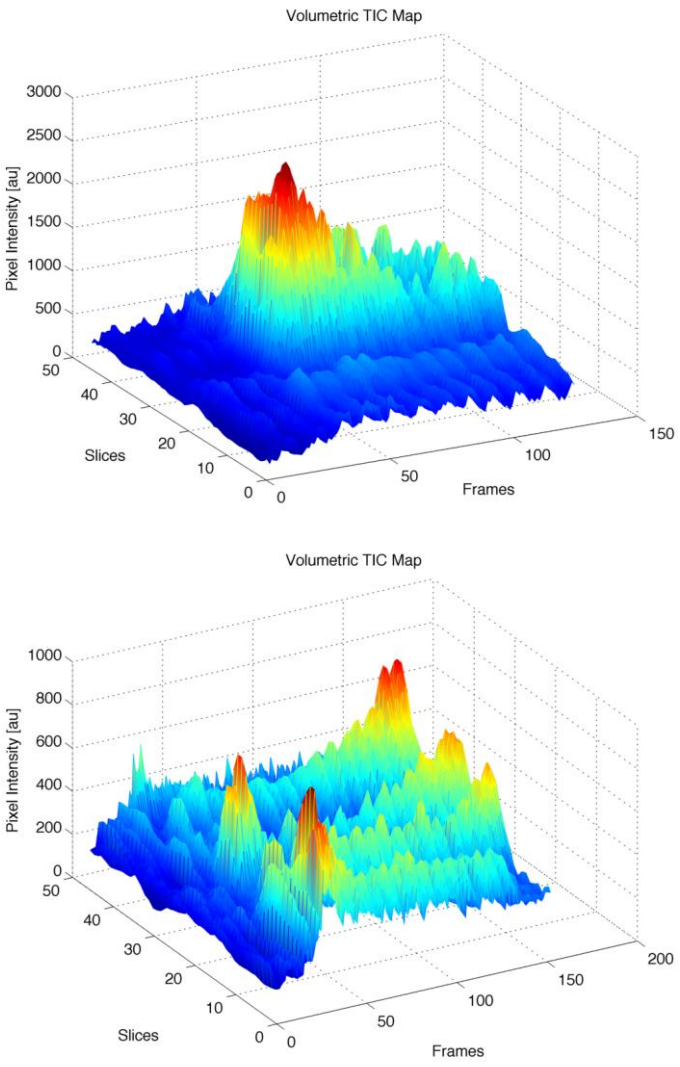

Figure 1. Signal intensity over contrast wash in and wash out at each individual slice from a benign lesion (top) and a case of high grade, invasive mammary carcinoma. Notice that the vascularity (shown by change in signal intensity) within the benign lesion is based primarily within a core region, while vascular locations are highly chaotic with a larger deviation in the malignant case.

This allows visualization of changes in signal intensity over the contrast agent wash in and wash out (an indicator of lesion vascularity). An example of this process is shown in Figure 1. A benign lesion with highly ordered, centralized flow is shown in the top

portion of Figure 1, while a malignant, invasive mammary carcinoma with chaotic vascularity throughout the lesion is shown in the bottom portion of the figure. When performing these algorithms over the entire dataset, we found that benign lesions showed a statistically significant difference in changes in signal intensity between the central and peripheral areas of the tumor (1.83 dB vs. 1.15 dB, $p < 0.001$), while no significant difference was observed between the central and peripheral regions in malignant lesions. These results are consistent with the concept that malignant lesions will have a highly unorganized, diffuse blood supply, while benign lesions are more likely to have structured, central vascularity. During the third year, we plan to continue to explore this concept as quantifiable diagnostic criteria for evaluating 3D subharmonic ultrasound of breast lesions.

Previously, we showed preliminary results of algorithms for parametric imaging of ultrasound contrast agent wash-in as a means for modeling blood flow kinetics (and thus being a useful quantifiable parameter for diagnosis) [1]. The project mentor (Dr. Forsberg) previously explored the use of contrast-enhanced ultrasound peak intensity to predict tumor angiogenic marker expression in two subcutaneous tumor models in rats. Such accurate predictive markers could offer a noninvasive technique for monitoring treatment response in breast cancer therapy. As part of the research portion of this project, the parametric algorithms in development for subharmonic imaging have since been applied to this existing dataset of contrast enhanced ultrasound exams of subcutaneous

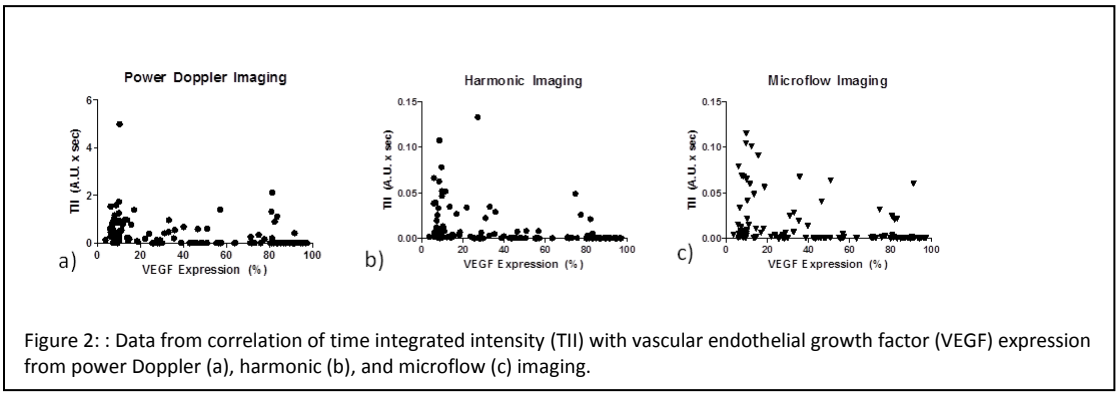

Figure 2: : Data from correlation of time integrated intensity (TII) with vascular endothelial growth factor (VEGF) expression from power Doppler (a), harmonic (b), and microflow (c) imaging.

tumors in rats. As part of the original study, breast tumor (NMU) or glioma (C6) cells were implanted in either the abdomen or thigh of 144 rats. After 6, 8 or 10 days, rats received a bolus injection of the ultrasound contrast agent Optison (GE Healthcare, Princeton, NJ; dose: 0.4 ml/kg) during power Doppler imaging (PDI), harmonic imaging (HI), and microflow imaging (MFI) using an Aplio ultrasound scanner with 7.5 MHz linear array (Toshiba America Medical Systems, Tustin, CA). While HI and PDI are standard imaging modes on most commercial ultrasound scanners, MFI is an experimental mode that uses destructive pulses followed by lower intensity imaging to generate cumulative maximum intensity images of microvasculature. Following imaging and animal sacrifice, tumors were stained for vascular endothelial growth factor (VEGF), Cyclooxygenase-2 (Cox-2), and the platelet endothelial cell adhesion molecule CD31). To evaluate the parametric code in this dataset, time-intensity curves of contrast wash-in

were constructed on a pixel-by-pixel basis and averaged to calculate maximum intensity, time to peak, perfusion, and time integrated intensity (TII) [3]. Significant correlation over the entire dataset (shown below in Figure 2) was only observed between TII and VEGF for all three imaging modes (R = -0.35, -0.54,- 0.32 for PDI, HI and MFI, respectively; $p < 0.0001$).

Tumor type and location affected these correlations, with the strongest correlation of TII to VEGF found to be with implanted C6 cells (R = -0.43, -0.54, -0.52 for PDI, HI and MFI, respectively; $p < 0.0002$). These results offered significant improvement in the overall correlation relative to the previous, static measurements used during the initial study. Thus, while results appear to be imaging mode and tumor type dependant, these algorithms being developed for lesion classification purposes may also be useful for monitoring treatment response in known malignant lesions.

As a secondary research focus, we have also begun to look at photoacoustic imaging as a potential means for monitoring treatment response in breast cancer therapy. Recently, the project mentor received funding to purchase a Vevo 2100 small animal ultrasound scanner with photoacoustic capabilities (through NIH S10 OD010408). Photoacoustics (PA) is an emerging imaging modality and these systems are now commercially available for preclinical research. Initially, light (generally in the 700-900 nm wavelength range) is directed through the tissue from a focused, tunable laser. As the light is absorbed, it generates thermal expansion which can then be detected using an ultrasound transducer. This technique benefits from the specificity of optical imaging and the resolution as well as increased penetration depths of ultrasound.

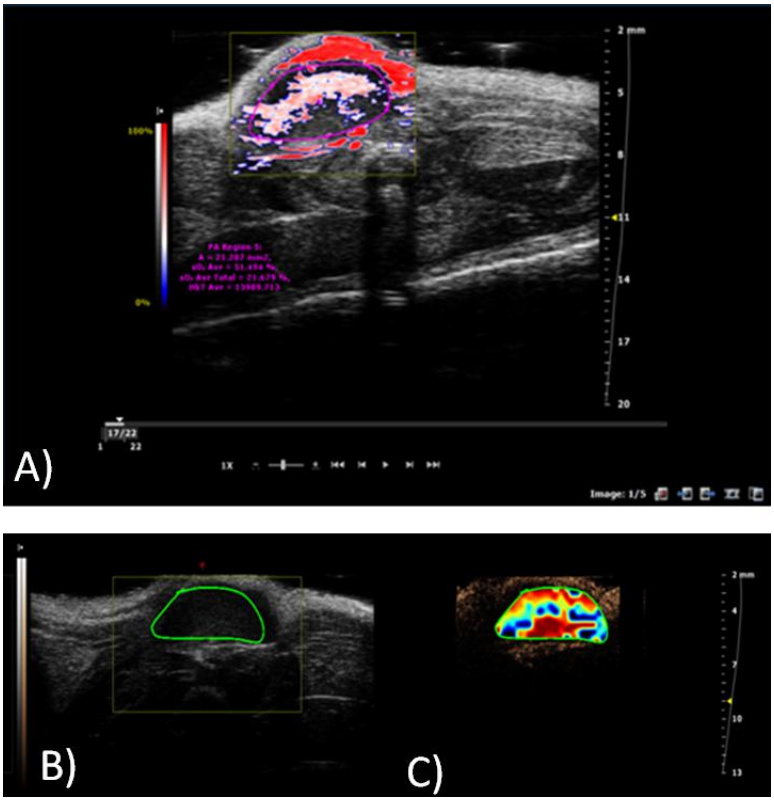

Figure 3. Imaging example with tumor ROI outlined. A: Representative image of a tumor in PA mode with high hemoglobin signal (13,989) and a relatively high SO2 Avg (51.5%). B: Corresponding CEUS image post contrast and C: peak enhancement parametric image.

MDA-MB-231 breast cancer tumors were implanted in the mammary pad of 11 nude rats. Ultrasound and PA scanning was performed using the Vevo2100 system. Contrast-

enhanced ultrasound (CEUS) was used to create maximum intensity projections as a measure of tumor vascularity. Photoacoustics were used to determine hemoglobin signal (HbT), oxygenation levels in detected blood (SO2 Avg), and oxygenation levels over the entire tumor area (SO2 Tot). An example of these images are shown in Figure 3, demonstrating strong photoacoustic signal (top) with quantitative oxygenation measurements, grayscale ultrasound of the tumor (bottom left), and the maximum intensity ultrasound contrast agent signal used as a measure of vascularity (bottom right). Tumors were then stained for VEGF, Cox-2, and CD31. Correlations between findings were analyzed using Pearson's coefficient. Significant correlation was observed between CEUS derived vascularity measurements and both PA indicators of blood volume ($R = 0.61$ for HbT, $R = 0.64$ for S02 Tot). However, no significant correlation was observed between these measurements and any of the immunohistochemical markers ($p > 0.18$). SO2 Avg showed significant inverse correlation with Cox-2 ($R = -0.65$, $p = 0.03$), but not with VEGF or CD31 ($p > 0.5$). While photoacoustically derived HbT and SO2 Tot may be a good indicator of tumor fractional vascularity, SO2 Avg appears to be a better predictor of Cox-2 expression. This research appears to indicate photoacoustics may be a better tool for monitoring breast cancer treatment response on the molecular level and may be a future area of research interest for the PI transitions into an independent faculty role.

6 KEY RESEARCH ACCOMPLISHMENTS

Training Component

- Continued observation of a large, multi-center breast imaging trial (data to be used for PI's research project)
- Observation of physician reading of mammograms, breast ultrasounds and breast MRI to better understand diagnostic thought process
- Attendance at Leading Edge in Diagnostic Ultrasound Breast Ultrasound Tutorial, American Institute of Ultrasound in Medicine 2013 Annual Meeting, as well as the 2013 World Molecular Imaging Congress.
- Attendance of Kimmel Cancer Center, Radiology, and Breast Cancer seminars/ case conferences

Research Component

- Developed and applied preliminary algorithms to measure blood flow kinetics based on temporal data for 4D subharmonic breast ultrasound exams.
- Applied these algorithms to an existing data set of contrast enhanced ultrasound exams in murine tumor xenografts to determine their ability to predict angiogenic marker expression
- Investigated spatial deviations in subharmonic derived blood flow parameters as a potential diagnostic criteria of malignancy
- Began preliminary studies into the use of photoacoustics as a potential means of evaluating breast tumor vascularity and immunohistochemisty in a murine model.

7 REPORTABLE OUTCOMES

Publications:

1. J.R. Eisenbrey, A. Marshall, D.A. Merton, J.B. Liu, T.B. Fox, A. Sridharan, F. Forsberg. Comparison of photoacoustically derived hemoglobin and oxygenation measurements with contrast enhanced ultrasound estimated vascularity and immunohistochemical staining in a breast cancer model. Submitted to Phys. Med. Biol, August ,2013.

2. J.R. Eisenbrey, C.C. Wilson, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Correlation of ultrasound contrast agent derived blood flow parameters with immunohistochemical angiogenesis markers in murine xenograft tumor models. *Ultrasonics*, 2013; 53:1384-91.

3. A. Sridharan, J.R. Eisenbrey, P. Machado, J.B. Liu, V.G. Halldorsdottir, J.K. Dave, H. Zhao, Y. He, S. Park, K. Wallace, K.E. Thomenius, F. Forsberg. Perfusion estimation using contrast enhanced three-dimensional subharmonic ultrasound imaging: an in vivo study. *Investigative Radiology,* 2013; 48:654-60.

Abstracts and Conference Proceedings:

1. J.R. Eisenbrey, D.A. Merton, J.B. Liu, T.B. Fox, A. Sridharan, F. Forsberg. Ultrasound contrast agent based vascularity measurements versus photoacoustic derived hemoglobin and oxygenation measurements in a breast cancer model. 2013 World Molecular Imaging Congress, P563.

2. J.R. Eisenbrey, C.C. Wilson, A. Sridharan, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Prediction of VEGF expression in two tumor models using dynamic contrast enhanced ultrasound: identification of optimal imaging mode and temporal parameter. 2013 World Molecular Imaging Congress, P241.

3. J.R. Eisenbrey, A. Sridharan, D. Merton, P. Machado, K. Wallace, C.L. Chalek, K. Thomenius, F. Forsberg. Four-dimensional subharmonic breast imaging: initial experiences. J. Ultrasound Med. 31:S18, 2013.

4. J.R. Eisenbrey, C.C. Wilson, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Correlation of ultrasound contrast agent-derived blood flow parameters with immunohistochemical markers in murine xenografts: influence of the imaging mode, tumor model, and subcutaneous location. J. Ultrasound Med. 31:S87, 2013.

5. A. Sridharan, J.R. Eisenbrey, F. Forsberg, V.G. Halldorsdottir, J.K. Dave, P. Machado, J.B. Liu, S. Park, S. Dianis, K. Wallace, K.E. Thomenius. In vivo perfusion estimation using 3D sub-harmonic ultrasound. *Prog. RSNA,* SSA21-05, 2012.

6. P. Machado, J.R. Eisenbrey, A. Sridharan, D.A. Merton, R.F. Mattrey, H. Ojeda-Fournier, K. Wallace, C.L. Chalek, K.E. Thomenius, F. Forsberg. Initial

experiences with 4D subharmonic breast Imaging. *Ultrasound Med Biol 2013*; 39(5): S27.

7. P. Machado, A. Sridharan, J.R. Eisenbrey, D.A. Merton, K. Wallace, C.L. Chalek, K.E. Thomenius, F. Forsberg. Method to improve visualization of vascularity using 4D subharmonic breast imaging. *Ultrasound Med Biol 2013*; 39(5): S27.

Scientific presentations:

1. J.R. Eisenbrey, D.A. Merton, J.B. Liu, T.B. Fox, A. Sridharan, F. Forsberg. Ultrasound contrast agent based vascularity measurements versus photoacoustic derived hemoglobin and oxygenation measurements in a breast cancer model. 2013 World Molecular Imaging Congress, Savannah, GA, September 2013.

2. J.R. Eisenbrey, C.C. Wilson, A. Sridharan, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Prediction of VEGF expression in two tumor models using dynamic contrast enhanced ultrasound: identification of optimal imaging mode and temporal parameter. 2013 World Molecular Imaging Congress, Savannah, GA, September 2013.

3. J.R. Eisenbrey, A. Sridharan, D.A. Merton, P. Machado, K. Wallace, C.L. Chalek, H. Ojeda-Fournier, R.F. Mattrey, K. Thomenius, F. Forsberg. 4D subharmonic breast imaging. The Leading Edge in Diagnostic Ultrasound Annual Conference. Atlantic City, NJ, May 2013.

4. J.R. Eisenbrey, A. Sridharan, D. Merton, P. Machado, K. Wallace, C.L. Chalek, K. Thomenius, F. Forsberg. Four-dimensional subharmonic breast imaging: initial experiences. AIUM Annual Meeting, New York, NY, April 2013.

5. J.R. Eisenbrey, C.C. Wilson, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Correlation of ultrasound contrast agent-derived blood flow parameters with immunohistochemical markers in murine xenografts: influence of the imaging mode, tumor model, and subcutaneous location. AIUM Annual Meeting, New York, NY, April 2013.

Awards and Honors:

1. Nominated for Best Poster Award, 2013 World Molecular Imaging Congress, Savannah, GA.

2. Student Travel Award for 2013 World Molecular Imaging Congress, Savannah, GA.

8 CONCLUSIONS

Work generated by this award has begun to shift from the training component and more towards the independent research phase. Preliminary algorithms and measures of quantification have been applied to the existing 4D breast SHI dataset and used as a potential indicator of malignancy. These algorithms have also been investigated as a potential sign of angiogenic marker expression. As the award progresses, the focus will be the refinement of these algorithms and their application to the larger clinical dataset.

9 REFERENCES

1. A. Sridharan, J.R. Eisenbrey, P. Machado, J.B. Liu, V.G. Halldorsdottir, J.K. Dave, H. Zhao, Y. He, S. Park, K. Wallace, K.E. Thomenius, F. Forsberg. Perfusion estimation using contrast enhanced three-dimensional subharmonic ultrasound imaging: an in vivo study. *Investigative Radiology,* 2013; 48:654-60.

2. F. Forsberg, R.J. Ro, T.B. Fox, et al., Contrast enhanced maximum intensity projection ultrasound imaging for assessing angiogenesis in murine glioma and breast tumor models: a comparative study. *Ultrasonics*. 51 (2011) 382-389.

3. J.R. Eisenbrey, C.C. Wilson, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Correlation of ultrasound contrast agent derived blood flow parameters with immunohistochemical angiogenesis markers in murine xenograft tumor models. *Ultrasonics*, 2013; 53:1384-91.